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09/246,451	02/09/1999	FRANCES H. ARNOLD	93731E827US1	6181

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EXAMINER
RAO, MANJUNATH N

ART UNIT	PAPER NUMBER
1652	14

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/246,451	Applicant(s) ARNOLD ET AL.	
	Examiner Manjunath N Rao	Art Unit 1652	

-- The MAILING DATE of this communication appears on th cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 November 2001* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-106,126-129,132 and 137-169* is/are pending in the application.

4a) Of the above claim(s) *1-106,126-129,132 and 137-145* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *146-169* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-106, 126-129, 132, 137-169 are still at issue and are present for examination. Claims 1-106, 126-129, 132, 137-145 remain withdrawn from consideration as being drawn to non-elected invention. Claims 146-169 are now under consideration.

Applicants' arguments filed on 11-5-01, paper No. 13, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 146-153 and 160 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 146-153 and 160 are drawn to "a cytochrome P450 oxygenase variant". However, it is not clear from the claim as written whether these are functional variants. It is well known in the art that a single amino acid change in certain enzymes can completely make the enzyme inactive. Amending the claim to recite the function would overcome this rejection.

Claim 154 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as

the invention. Claim 154 recites the phrase "variant of the variant". It is highly unclear to the Examiner as what applicants mean by the above phrase.

Claim 161 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 161 is drawn to a oxygenase variant having at least 10 times the catalytic activity of the wild-type enzyme identified by following the steps a), b) and c). However, without comparing the activity levels of the variant with that of the wild type enzyme it is unclear to the Examiner as to how applicants can conclude that a variant is 10 times as active as the wild type. Amending the claim to include another step such as "d) and comparing the activity level with the activity levels of the wild type under similar reaction conditions and selecting a variant enzyme with an activity level at least 10 times greater than that of the wild-type enzyme" or the like would overcome this rejection.

Claim 164 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 164 is drawn to a oxygenase variant having at least 10 times the stability of the wild-type enzyme identified by following the steps a), b) and c). However, without comparing the activity levels of the variant with that of the wild type enzyme it is unclear to the Examiner as to how applicants can conclude that a variant is 10 times as stable as the wild type. Amending the claim to include another step such as "d) and comparing the stability level with the stability of the wild type under similar reaction conditions and selecting a variant enzyme

with a stability at least 10 times greater than that of the wild-type enzyme" or the like would overcome this rejection.

Claim 167 and claims 168-169 which depend from claim 167 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 167 is drawn to a oxygenase variant comprising specific mutations at amino acid positions 331, 242, and 280. However, applicants claim that such a mutant was identified by performing the activity assays comprising the steps a), b), and c). It is not clear to the Examiner as to how one skilled in the art would be able to identify variants or mutants with changes at specific amino acid positions just by doing activity assays.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 146-159, 161-166 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a mutant cytochrome P450 oxygenase isolated from *Ps.putida* in which a glutamic acid at position 331 is changed to lysine, an arginine at position 280 is changed to lysine and a cysteine at position 242 is changed to phenylalanine, does not reasonably provide enablement for any or all other mutant/variant cytochrome P450 oxygenase enzyme from any source. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. See previous Office action for rejection.

In response to the previous Office action, applicants argue that the rejection under 35 U.S.C. §112, first paragraph is not proper because the specification teaches the complete nucleotide and amino acid sequences of the oxygenase with SEQ ID NO:2, protocols for using that sequence information, and protocols for testing for enzymatic activity, and methods for producing variants of a disclosed sequence are within the skill of the ordinary artisan. This is not persuasive because new claims (146-153) are drawn to a variant comprising a mutation at a position *corresponding* to amino acid 331, 280 or 242 of cytochrome $P450_{cam}$ from *P.putida*. Thus it appears that claims are drawn to any cytochrome P450 with a mutation at the *corresponding position* of SEQ ID NO:2. While the specification is probably enabled for variant cytochrome P450 which are highly similar to cytochrome $P450_{cam}$ with SEQ ID NO:2 (for example cytochrome P450 whose sequence homology is in the range of 95-99% to SEQ ID NO:2) the specification is clearly not enabling for any variant which is not structurally related to cytochrome P450 by such high structural homology. Applicants have not taught as to how one skilled in the art would go around finding out or making amino acid change *corresponding to* amino acids 242, 280 or 331 in any cytochrome P450. Furthermore, even if applicants produced such highly similar sequences they have not shown expectation of similar effects. Furthermore, while methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, etc. are well known to the skilled artisan, producing variants as claimed by applicants (i.e., with 2-10 times the activity and stability of the wild type) requires that one of ordinary skill in the art know or be provided with guidance for the selection of which of the infinite number of variants have the claimed property. The oxygenase enzyme in question has 414 amino acids. Applicants claim that changing any one amino acid among the total of 414

amino acids would yield the above variant. Without guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. This would clearly constitute undue experimentation. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has not been provided in the instant specification. As previously stated the specification does not establish: (A) a rational and predictable scheme for isolation and characterization of any variant cytochrome P450 oxygenase from SEQ ID NO:2 with an expectation of obtaining the desired biological activity and function; (B) the general tolerance of cytochrome P450 oxygenase (with SEQ ID NO:2) enzyme to modification and extent of such tolerance; and (C) the specification provides insufficient guidance as to which of the infinite possible choices is likely to be successful. Hence the rejection is maintained.

Claims 146-166 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 146-166 are directed to polypeptide cytochrome $P450_{cam}$ oxygenase variants with specific amino acid changes and variants which are at least two to ten times as active and/or as stable as the wild type cytochrome $P450_{cam}$ with SEQ ID NO:2. Claims 146-166 are rejected under this section of 35 USC 112 because the claims are directed to a genus of

polypeptides including modified polypeptide sequences, modified by at least one of deletion, addition, insertion and substitution of an amino acid residue in SEQ ID NO:2 and fragments of SEQ ID NO:2 that have not been disclosed in the specification. No description has been provided of all the modified polypeptide sequences encompassed by the claim. No information, beyond the characterization of a single mutant with changes to amino acids at three specific positions in SEQ ID NO:2 has been provided by applicants which would indicate that they had possession of all the claimed genus of modified polypeptides. The specification does not contain any disclosure of the structure and function of all the polypeptide sequences derived from SEQ ID NO:2 or sequences corresponding to it, including fragments and variants within the scope of the claimed genus. The genus of polypeptides claimed is a large variable genus including peptides which can have a wide variety of structures and with the potentiality of different levels of activity and stability. Therefore many structurally and functionally unrelated polypeptides are encompassed within the scope of these claims. The specification discloses only three specific variant species of the claimed genus which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus. Therefore, one skilled in the art cannot reasonably conclude that applicant had possession of the claimed invention at the time the instant application was filed.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

In response to the previous Office action, applicants have traversed the above rejection arguing that the enzymes of the claimed invention all have the same unifying function,

oxygenase activity, and such a function and assays are well described in the art. Applicants also argue that cytochrome P450 oxygenases is a particularly will-defined group with known structural features. Applicants also argue that new claims correlate to the structural and functional features of the variant with those form *P.putida* providing a reference point and that a variant cytochrome P450 could be aligned with wild type using an algorithm. However, Examiner respectfully disagrees. While one skilled in the art can align sequences using algorithms, applicants have not described the structure and function of all such variants and claims are also not limited to cytochrome P450s with structural homology to the cytochrome P450 of SEQ ID NO:2. The enzyme in question has 414 amino acids. The structures of all the variants that results from replacing each amino acid with any one of the other 19 naturally occurring amino acids results in an extremely large number of amino acid sequences. Applicants have not provided the structure and/or any other identifying characteristics or properties of all those sequences which now encompassed by the claimed invention. Applicants have also not provided the function of all such variants wherein one or more than one amino acid has been changed. The scope of the claim encompasses all variants that can be derived by changing or replacing any or all of the 414 amino acids with 19 other amino acids and Applicants have not described a representative number of them. Hence the rejection is maintained.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 155-159 are rejected under 35 U.S.C. 102(b) as being anticipated by Manchester(a)et al. Protein Engineering, 1995, Vol 8(8):801-807 or Manchester(b) et al. (Biochemie, 1996, Vol. 78(8-9), 714-722). This rejection is based upon the public availability of printed documents. Claim 155-159 of the instant application are drawn to a cytochrome $P450_{cam}$ oxygenase variant from *P.putida* encoded by a first polynucleotide that hybridizes to a second polynucleotide wherein the second polynucleotide encodes the cytochrome $P450_{cam}$ oxygenase with the mutation at position 331 or 242 or 280. Claims are also drawn to the variant enzyme having a catalytic activity that is 2-10 times that of the wild type enzyme and variant enzyme having a stability that is 2-10 times that of the wild type enzyme. Manchester (a) et al. and Manchester (b) et al. disclose such a variant cytochrome P450 enzyme. Since there is no limitation placed on the position of amino acid and the number of changes that can be present in the amino acid sequence for a variant cytochrome P450 oxygenase, above claims read on the variant enzyme disclosed by Manchester(a) or Manchester(b) et al. Even though Manchester (a) et al. or Manchester(b) et al. do not disclose the variant enzyme as having a catalytic activity that is 2-10 times that of the wild type enzyme or having a stability that is 2-10 times that of the wild type enzyme Examiner also takes the position that such characteristics are inherent of the disclosed variant and hence read on the above claims.

Since the Office does not have the facilities for examining and comparing applicants' protein with the protein of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the protein of the prior art does not possess the same material structural and functional

characteristics of the claimed protein). See *In re* Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re* Fitzgerald et al., 205 USPQ 594.

In response to the previous Office action, applicants have traversed the above rejection arguing that the above reference is concerned with dehalogenation properties of a single mutant and does not teach anything about the stability or enzyme activity in promoting oxygenation of a substrate. Furthermore, applicants also argue that it is unknown whether the enzyme in the reference has any oxygenation activity at all, since the dehalogenation reaction can only take place in the complete absence of oxygen. Examiner respectfully disagrees with such an argument. Even though Manchester et al. (a or b) does not assay for the oxygenase activity, they do not specifically state anywhere in their reference that the oxygenase activity was lost in their mutant. Oxidation/reduction is the primary activity of all cytochrome P450 enzymes. Furthermore, the argument that the variant enzyme in the reference may not have oxygenation activity at all because the reference states that dehalogenation takes place in the absence of oxygen is misplaced. This is because, the reference clearly states that "in the absence of oxygen, cytochrome P450 can reductively dehalogenate halogenated hydrocarbons", meaning that this is an additional property of cytochrome P450 systems that can be exploited in the absence of oxygen. While Examiner agrees that Manchester et al. references do not disclose any of the specific mutations set forth in claims 146-153, 160, 167-169, the reference anticipates claims 155-159. Therefore the above rejection is maintained.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, THIS ACTION IS MADE FINAL. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Manjunath Rao whose telephone number is (703) 306-5681. The Examiner can normally be reached on M-F from 6:30 a.m. to 3:00 p.m. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, P.Achutamurthy, can be reached on (703) 308-3804. The fax number for Official Papers to Technology Center 1600 is (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

PONNATHAPU ACHUTAMURTHY
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Manjunath N. Rao. Ph.D.
February 21, 2002